

152

INFLUENCE OF SOME OSTEOTROPIC FACTORS ON MODULATION OF THE RANKL2 ISOFORM

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Purpose: Bone homeostasis is controlled by some members of the TNF family, of which RANKL is an essential factor for mediating bone resorption. RANKL exists as three isoforms, RANKL1, 2, and 3, and very little is known about their regulation and modulation. A recent study by our group on human normal and osteoarthritic subchondral bone osteoblasts [1] showed that these cells differentially express membranous RANKL as well as RANKL1 and RANKL3 isoforms, and that RANKL is differentially regulated by some osteotropic factors. In that study, specific human RANKL2 cDNA primers could not be designed, thus its expression could not be determined. Here we investigated the RANKL2 protein modulation upon treatment with the osteotropic factors TNF- α , vitamin D₃ and PTH through a mechanistic model, i.e. human cells that do not constitutively produce RANKL but were stably transfected with cDNA of the RANKL2 isoform.

Methods: Human embryonic kidney 293 cells, which do not express RANKL, were stably transfected with the cDNA encoding the transmembranous and extracellular domain of murine RANKL. Semi-quantitative PCR and Western Blot were used to determine the level of RANKL2 on the 293 and 293 RANKL2 cells. For the modulation experiments, the 293 RANKL2 cells were incubated for 72 hours with TNF- α (5 ng/ml), vitamin D₃ (50 nM), or PTH (100 nM). For protein stability determination, cells were pretreated with or without actinomycin D (5 μ g/ml) for 1 hour and then incubated (0-24 hours) with the above factors with or without actinomycin D. The production of RANKL2 protein was determined in the cell lysates by a specific DUOset ELISA.

Results: Expression and production analyses confirmed that the 293 RANKL2 cells strongly expressed and produced RANKL2. Data showed that TNF- α significantly increased ($p < 0.03$) RANKL2 production, but vitamin D₃ and PTH had no effect. Treatment with vitamin D₃ and PTH in conjunction with actinomycin D had no effect on the stability of the RANKL2 protein. However, TNF- α plus actinomycin D increased the stability of RANKL2 by increasing its half-life, and statistically significant difference ($p < 0.005$) between actinomycin D alone and actinomycin D plus TNF- α was reached at a 4-hour incubation period.

Conclusions: Our study brings to light that the RANKL2 isoform can be modulated by TNF- α , a factor highly implicated in some diseases involving bone resorption and an increase in RANKL activity, such as rheumatoid arthritis. Interestingly, other osteotropic factors, vitamin D₃ and PTH, did not modulate RANKL2 protein production or its stability/half-life, which could reflect the differential effects of these factors in bone remodelling. These findings, in addition to the previous study [1], reveal that all three RANKL isoforms can be modulated, which strongly suggests that modulation of a specific RANKL isoform may be useful for a possible classification of certain osteolytic diseases and/or to assist in identifying specific therapeutic treatments.

References

[1] Tat SK, et al. *Bone* 2008;43:284-91.

153

MODULATION OF THE PHENOTYPE OF HUMAN OSTEOARTHRITIC SUBCHONDRAL OSTEOBLASTS BY CONJUGATED LIPIDS

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Purpose: Altered bone remodeling is important in the initiation of osteoarthritis (OA). This may be due to the abnormal phenotype markers and proliferation of human OA osteoblasts (Ob). The canonical Wnt/ β -catenin signaling pathway is crucial for osteogenesis and we previously demonstrated that it is reduced in OA. n-3 or n-6 polyunsaturated fatty acids (PUFAs) affect bone health status and can modify cell markers. Moreover, recent data suggest bone marrow lesions, which are linked with OA, are also more prominent in presence of n-6 PUFAs or monounsaturated lipids.

Here we questioned if alterations of lipids in OA Ob could alter their phenotype, Wnt/ β -catenin signaling, and mineralization.

Methods: We prepared primary human subchondral osteoblasts from tibial plateaus of OA patients undergoing total knee arthroplasty. OA Ob were incubated for a week in presence of 50 μ g/ml of oleate (C18:1), linoleate (C18:2, n-6 PUFA), linolenate (C18:3, n-3 PUFA), or vehicle. We evaluated the role of these lipids on alkaline phosphatase and osteocalcin expression and production, prostaglandin E₂ (PGE₂) production, and mineralization. We determined expression of selected genes by qRT-PCR, alkaline phosphatase activity by substrate analysis, osteocalcin levels using a selective EIA, and PGE₂ using an ELISA. Wnt3a-dependent Wnt signaling was measured using the TOPflash TCF/lef reporter assay. Mineralization in presence of the different lipids was assessed by Alizarin red staining in response to 10 ng/ml BMP-2, 10 ng/ml Dexamethasone (Dex), or their combination.

Results: Lipids modified the expression and production of alkaline phosphatase and osteocalcin in OA Ob. Oleate increased alkaline phosphatase whereas linolenate decreased it. Oleate and linoleate both increased osteocalcin release whereas linolenate decreased it as for alkaline phosphatase activity. Oleate and linoleate both triggered PGE₂ production in normal Ob to levels observed in high OA Ob, and these treatments increased PGE₂ levels in both low and high OA Ob to similar levels. In contrast, linolenate did not alter PGE₂ production by normal and low OA Ob yet reduced it in high OA Ob. Wnt3a-dependent Wnt signaling remained similar amongst the different lipid additions. While neither BMP-2 nor Dex alone modified significantly the mineralization of OA Ob, their combination enhanced it. Oleate slightly increased the mineralization potential of OA Ob in presence of BMP-2 and Dex whereas linoleate and linolenate did not modify significantly the response of OA Ob to combined BMP-2 and Dex treatments.

Conclusions: These results suggest that acute treatment with monounsaturated lipids and n-6 PUFAs can exacerbate the altered phenotype of OA Ob and modify that of normal Ob. Monounsaturated and n-6 PUFAs also contributed to increase PGE₂ production in normal and OA Ob while n-3 PUFAs stabilized PGE₂ production. Surprisingly, monounsaturated lipids could also improve the mineralization potential of OA Ob. In contrast, n-3 PUFAs improved the phenotype of OA Ob while having modest effect on mineralization. Hence, an altered lipid environment and/or metabolism in OA bone tissue could contribute to the abnormal phenotype of osteoblasts and to alter their mineralization capacity.

154

ARTICULAR CARTILAGE RESPONSE TO IL-1 β IS MODULATED BY THE UNDERLYING BONE REMODELING ACTIVITY: A NEW DEMONSTRATION OF BONE AND CARTILAGE INTERACTION

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Purpose: Osteoarthritis involves cartilage and bone changes although the role of bone in the pathophysiology is still unclear. We have previously shown that inhibition of bone resorption had a protective effect on osteoarthritis induced in mice with high bone remodelling, suggesting that level of bone remodelling might influence cartilage damage. The aim of this study was to evaluate the effect of bone remodelling activity on cartilage degradation ex vivo in human explants.

Methods: explants of bone and cartilage were harvested from healthy zones in patients undergoing total replacement therapy for unicompartmental knee OA. Bone and articular cartilage were separated manually and cultured in parallel in red-phenol-free medium. Bone explants were cultured BGJb, and cartilage in DMEM. After 24h of stimulation with IL-1 β (10ng/ml), bone explants were cultured in presence of estradiol at 10 or 1000 nM or pamidronate at 1 μ M for 72h. After changing the medium for 24h, bone culture supernatant was transferred on cartilage explants previously stimulated by IL-1 β for 24h (ratio 1:3). Control cartilage explants were cultured with the same ratio of BGJb. Estradiol and pamidronate was also directly cultured with cartilage explants to test a possible direct effect of these drugs on cartilage catabolism. Proteoglycan (using the dimethyl-methylene blue colorimetric assay) and aggrecan neoepitops (by western blotting (WB) targeting the ARGSV sequence) release in the supernatant were measured as cartilage catabolism markers.

Results: IL-1 β on human cartilage explants induced an increase in proteoglycan release ($124 \pm 2.4\%$ $p = 0.037$) when compared to controls. Supernatant